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Identification and Quantification of Phenolic Compounds in Bambangan (*Mangifera pajang* Kort.) Peels and Their Free Radical Scavenging Activity

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ABSTRACT: Phenolic compounds and antioxidant capacity of acidified methanolic extract prepared from fully ripe bambangan (*Mangifera pajang* K.) peel cultivated in Sarawak, Malaysia, were analyzed. The total phenolic content (98.3 mg GAE/g) of bambangan peel powder (BPP) was determined by the Folin−Ciocalteu method. BPP showed a strong potency of antioxidant activity and was consistent with that of BHT and vitamin C as confirmed by the DPPH (1,1-diphenyl-2-picrylhydrazyl) radical scavenging activity and FRAP (ferric-reducing antioxidant power) assays. Gallic acid, *p*-coumaric acid, ellagic acid, protocatechuic acid, and mangiferin were the major compounds among the 16 phenolics that have been identified and quantified in *M. pajang* peels with 20.9, 12.7, 7.3, 5.4, and 4.8 mg/g BPP, respectively. Peak identities were confirmed by comparing their retention times, UV−vis absorption spectra, and mass spectra with authentic standards. The 16 phenolic compounds identified in *M. pajang* K. using HPLC-DAD and TSQ-ESI-MS are reported here for the first time.

KEYWORDS: *Mangifera pajang*, peel, phenolic compounds, scavenging activity, HPLC−ESI-MS

INTRODUCTION

Bambangan (*Mangifera pajang* Kort.) is an underutilized fruit that is found in Malaysia (Sabah and Sarawak), Brunei, and Indonesia (Kalimantan).[1] The fruit is 3 times larger than commercial mango (*Mangifera indica*). Its peels constitute approximately 27% of the fruit, compared to 15% for commercial mango (*M. indica*).[2] Bambangan peels are available in large amounts as industrial byproduct and have been shown to contain large amounts of valuable compounds such as polyphenols that could be exploited. To date, fresh consumption of bambangan fruit is less popular because of its fibrous flesh and thick peel; hence, alternative products from this fruit are desirable. Using the fruit peels as food or food ingredients can be done by either soaking the fresh peels in sweet solution and using it as marmalade or incorporating the powdered peel with food products (biscuits, macaroni, etc) to improve the nutraceutical properties of these products.

Mango, a member of the plant family Anacardiaceae, is one of the most important tropical fruits in the world. It is listed among the top of superfruit list [3] because of its health-promoting properties. Peels and seeds are the major byproducts generated during the processing of mango, amounting to 35−60% of the total fruit weight.[4] Because the disposal of these byproducts has become an issue, various attempts to utilize seed kernels and peels have been made over the past few decades. Ajila et al.[2] reported that mango peels contain valuable compounds such as polyphenols, carotenoids, and vitamins. In the past decade, interest in the study of mango phenolics has increased. Phenolics have potent antioxidant activity and are believed to have health-promoting properties that make the consumption of fruits and derived processed products from fruit pulp, peels, and seed kernels a very healthy habit.[5,6] Recent epidemiological studies have demonstrated that the increased consumption of fruit and vegetables is associated with a reduced risk of cardiovascular disease and certain types of cancer because of their content of antioxidants such as phenolic compounds.[7,8]

Separation techniques such as high performance liquid chromatography (HPLC) have been used in many applications in the study of food components. The analysis of complex food extracts requires highly selective analytical techniques to characterize unknown compounds. The coupling of chromatography to mass spectrometry (MS) yields a powerful instrument for the rapid and accurate identification of the chemical constituents of plant extracts. This instrument overcomes the major analytical issue with HPLC, which is the scarce information yielded by the detectors usually associated with HPLC, such as UV−vis detectors. The high analytical power of online LC−MS has been convincingly shown by others.[9,10]

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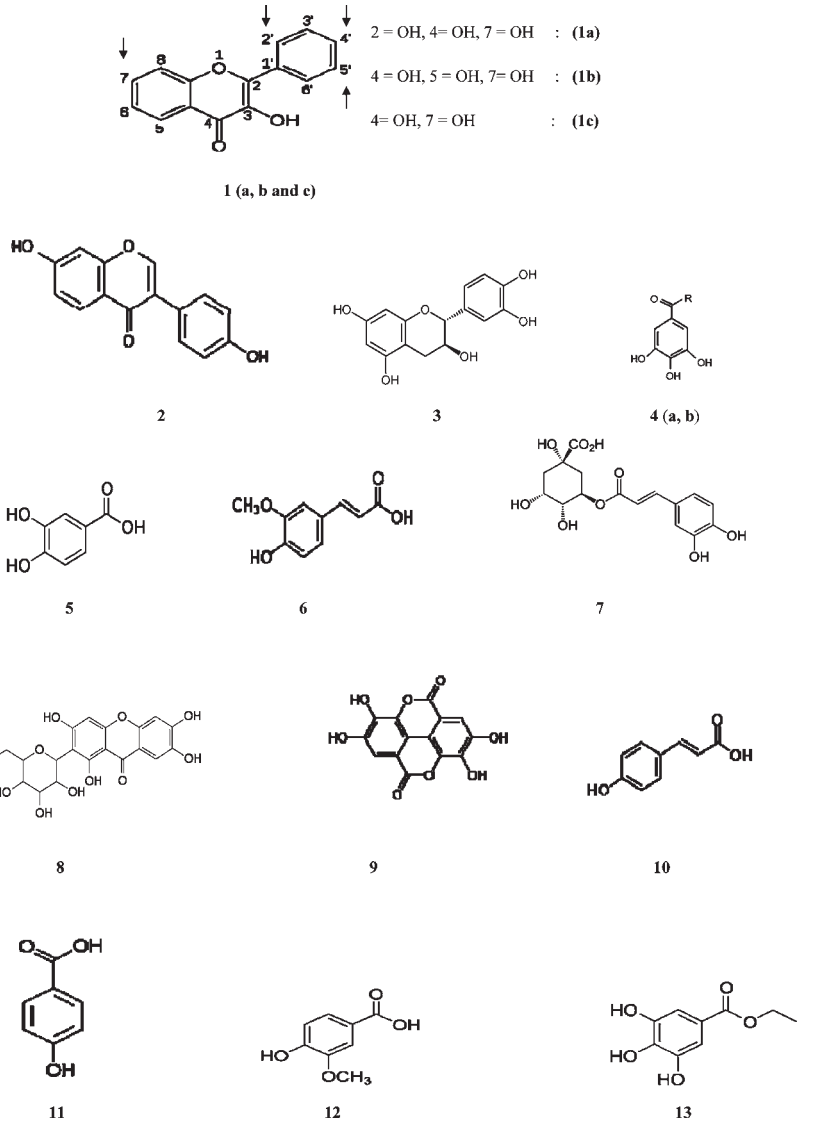

Figure 1. Compounds separated from *Mangifera pajang* K. peel extract: morin (**1a**), rutin (**1b**), kaempferol (**1c**); daidzein (**2**); (+)-catechin (**3**); gallic acid (R = OH) (**4a**), methyl gallate (R = CH_3) (**4b**); protocatechuic acid (**5**); ferulic acid (**6**); chlorogenic acid (**7**); mangiferin (**8**); ellagic acid (**9**); *p*-coumaric acid (**10**); 4-hydroxybenzoic acid (**11**); vanillic acid (**12**); ethyl gallate (**13**).

The quantitative determination of the free phenolic content of an extract is important for the estimation of their phenolic content and for quality control purposes. In the current study, we report the separation, structural characterization, and the quantitative determination of the phenolic constituents from the methanol soluble portion of *M. pajang* peels.

Similar studies have been conducted using *M. indica*. To date, there is little information on the valuable phytochemical compounds found in *M. pajang* peels, their free radical scavenging activity, and their health-promoting properties. A few investigations have been conducted on the proximate composition and the possible utilization of bambangan pulp and juice powder,[11] study of dietary fiber from fruit pulp,[12] and the isoflavone content of the flesh;[13] however, studies on the composition of the peels are very limited.

Commercial mango peels are known to contain a mixture of polyphenols as phenolic acid, flavonoids, and xanthones. Of these, the most abundant compounds that have been identified in *M. pajang* extract are presented in Figure 1.

This study aimed to evaluate the identity and the quantity of the phenolic compounds found in extracts of *M. pajang* fruit peels by utilizing HPLC-DAD. Phenolics were identified and quantified by comparing their retention times and characteristic UV−vis absorption spectra to authentic standards, and identifications were supported by LC−ESI-MS analysis.

MATERIALS AND METHODS

Chemicals. All standards used for identification and quantification were purchased from Sigma Aldrich Co. (St. Louis, MO, USA) and were as follows: gallic acid, protocatechuic acid, *p*-coumaric acid, chlorogenic acid, ellagic acid, vanillic acid, 4-hydroxybenzoic acid, ferulic acid, methyl gallate, ethyl gallate, (+)-catechin, mangiferin, morin, rutin, daidzein, and kaempferol. 2,4,6-Tripyridyl-*s*-triazine (TPTZ), 1,1-diphenyl-2-picrylhydrazyl (DPPH), and butylated hydroxytoluene (BHT) were purchased from Fisher Scientific (Loughborough, U.K.). The Folin−Ciocalteu reagent was obtained from Merck (Darmstadt, Germany).

HPLC grade solvents were also obtained from Fisher Scientific (Loughborough, U.K.).

Standard Curve Preparation. The standard stock solutions were prepared by dissolving standards in methanol to 100 μg/mL. For the calibration curves, four additional concentrations (20, 40, 60, and 80 μg/mL) were prepared by the dilution of the stock solutions with methanol.

Sample Preparation. Bambangan fruits were obtained from Sarawak, Malaysia, in October 2009. The fully ripened bambangan peels were collected immediately after the fruit was peeled. After being blanched at 83 °C for 5 min to avoid the browning reaction, the thick peels were mixed with water (1:2 w/v) for easier blending using a CB10BT blender (Waring Commercial, Torrington, USA). The peel mixture was then spread out on trays to accelerate drying at 50 °C ± 2 °C for 18 h to bring the moisture content to 4%. The dried peels were comminuted using an Ultra centrifugal mill (Zm100, Retsch, Haan, Germany) and passed through a 250 μm sieve to obtain desired particle sizes. The bambangan peel powder (BPP) was then stored at 23 °C in a tightly sealed plastic container.

Total Polyphenol Assay (TPA). Aliquots of BPP (500 mg) were placed into 100 mL volumetric flasks containing 50 mL of 4% hydrochloric acid (HCl) in methanol. The final volume was brought to 100 mL with the same solution. Total polyphenols were estimated colorimetrically by the Folin−Ciocalteu method as described previously,[14−16] modified by replacing acetonitrile with methanol. The procedure was clearly descried.[17] Methanol was used as blank, and reagents were used as controls. Quantification was based on a standard curve established with 20, 40, 60, 80, and 100 μg/mL gallic acid. Results were expressed as milligram of gallic acid equivalents (GAE) per gram of BPP.

DPPH Assay. The radical scavenging activity (RSA) of BPP extracts was determined using the free radical DPPH$^{\bullet}$ (100 μM in methanol). Monitoring of the depletion of DPPH$^{\bullet}$ was streamlined by measuring the absorbance of samples at 517 nm. The assay was performed using a microplate reader (UVM 340 microplate reader, Austria) according to the procedure described in our previous work.[17] The percentage inhibition value was calculated according to the following equation:

$$\text{scavenging activity } (\%) = 100 - \left[\frac{A_s}{A^0} \times 100\right]$$

where A_s is the absorbance of the sample and A^0 is the absorbance of the blank control.

The percentage of inhibition was plotted against the concentration range from 50 to 250 μg/mL of sample. The IC_{50} was determined from the absorbance plots as the concentration of sample extract required for 50% of the DPPH radical to be scavenged over the specified time.

Ferric-Reducing Antioxidant Power (FRAP) Assay. The procedure was conducted according to the method previously described.[18] The FRAP reagent was prepared freshly by mixing 300 mM acetate buffer (pH 3.6), 10 mM 2,4,6-tripyridyl-*s*-triazine (TPTZ) solution, and 20 mM $FeCl_3 \cdot 6H_2O$ in a ratio of 10:1:1. The reagent was warmed to 37 °C in water bath. The absorbance was read at 593 nm (A_1) against deionized water as blank.

Different concentrations ranged from 50 to 400 μg/mL BPP extract, and vitamin C as a positive control were prepared, dissolved in 10 mL of distilled water. Then 50 μL of each sample or positive control and 150 μL of deionized water were added to 1550 μL of FRAP reagent. After addition of the sample to the FRAP reagent, a second absorbance at 593 nm was read after 8 min (A_2). Finally A_1 was deducted from A_2 to determine the FRAP value of the sample. A standard curve was plotted with different concentrations (with range 100−1000 μM) of $FeSO_4 \cdot 7H_2O$. The final result was expressed as the concentration of antioxidants having a ferric reducing ability equivalent to that of 1 μM $FeSO_4 \cdot 7H_2O$.

Preparation of the Extract for Chromatographic Analysis. To obtain large amounts of phenolic compounds, hydrolysis reaction was conducted. The extract was prepared following the method described earlier,[19] with some modifications. Briefly, 500 mg of BPP was placed in a test tube and extracted with 40 mL of acidified methanol (4 mL of HCl was added to obtain a final concentration of 1.2 M) and 2 mg of ascorbic acid as antioxidant. Samples were shaken at 240 rpm on an orbital shaker at 35 °C for 16 h. After the mixture was centrifuged at 1500*g* for 10 min, the supernatants were collected and brought to a final volume of 50 mL with deionized water. The extract was stored at −80 °C. Before use, the extract was filtered through a 0.45 μm nylon membrane filter (SRP 15, Machery Nagel, Düren, Germany) and then analyzed by HPLC.

Linearity. The HPLC method was validated in terms of the linearity of calibration curves that have been previously determined for the standards and the components (1−13) of interest in the BPP extract (Figure 1). The linearity of the detector response was determined using five concentrations, with three injections for each concentration. A linear relationship between the peak area and the concentration (20−100 μg/mL) was observed for each standard with correlation coefficient ranges from $r = 0.9912$ to $r = 0.9993$.

Reproducibility. The reproducibility of the injection integration procedure was determined for standards and for the components of BPP. The solutions were prepared three times, and each solution was injected three times. The relative standard deviations for the replicate injections were calculated (gallic acid, 0.8%; protocatechuic acid, 1.6%; (+)-catechin, 1.7%; chlorogenic acid, 3.9%; methyl gallate, 4.3%; mangiferin, 0.9%; 4-hydroxybenzoic acid, 4.6%; vanillic acid, 2.6%; ethyl gallate, 5.0%; *p*-coumaric acid, 1.1%; ferulic acid, 2.3%; rutin 1.0%; ellagic acid, 1.3%; morin, 2.0%; daidzein, 2.4%; and kaempferol, 2.7%). The relative standard deviation of retention times was less than 1.0%.

Equipment and Chromatographic Conditions. Quantitative analyses of the BPP extracts were performed on an Agilent series 1100 HPLC system (Agilent Technologies, Palo Alto, CA, USA) equipped with a diode array detector (DAD) following the method previously described.[20] Briefly, chromatographic analyses were performed using a Lichrospher C18 5 μm (250 mm × 4 mm, i.d.) column purchased from Merck (Darmstadt, Germany). The mobile phase consisted of 0.5% (v/v) acetic acid in water (mobile phase A) and 100% methanol (mobile phase B) at a flow rate of 0.8 mL/min with a gradient elution program and a 30 min run time. The gradient elution began at 100% phase A with a linear decrease to 10% at 20−25 min. Over the next 5 min, phase A increased back to 100%. The injection volume was 20 μL. Analyses were performed at 25 °C. The majority of the phenolic compounds in bambangan peels in HPLC chromatogram showed a UV−vis spectral result at 280 nm as mangiferin and phenolic acids, gallic acid, *p*-coumaric acid, ellagic acid, protocatechuic acid, vanillic acid, etc. The other phenolic compounds as morin, daidzein, and kaempferol were clearly shown at 254, 320, or 365 nm.

The analysis of the phenolic compounds found in BPP by electrospray ionization mass spectrometry (ESI-MS) was performed using an Applied TSQ Quantum Ultra- LCMS (Thermo Fisher, San Diego, CA, USA). The mass spectrometer was operated in both negative and positive electrospray ionization (ESI±) modes, and a maximum of 3000 Da was used for the best resolution. The spray voltage was 3500 V. The sheath/auxiliary/sweep gas was 99% pure nitrogen, and the sheath gas pressure was 30 psi with 5 psi for the auxiliary gas pressure. The capillary temperature was 270 °C. The injection volume was 10 μL, and the flush speed was 100 μL/s.

The surveying method used for LC−MS was as follows: the run time was 30 min with a 1 s rise time. The scan wavelength was set at 200−600 nm. The scan bandwidth was set at 1 nm with a scan rate of 5 (Hz) and a scan step of 1 nm. The channel sample rate was set at 10 (Hz) with the three following channels: for channel A, the wavelength was 214 nm and the bandwidth was 9 nm; for channel B, the wavelength was 254 nm and the bandwidth was 9 nm; for channel C, the wavelength was

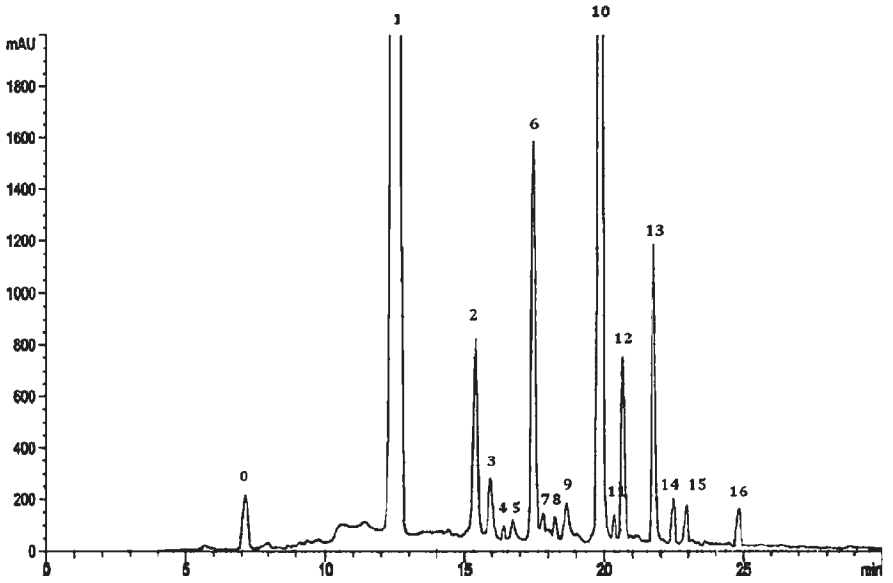

Figure 2. HPLC profile of phenolic compounds in *Mangifera pajang* peel, peak identification: 0, unknown; 1, gallic acid; 2, protocatechuic acid; 3, catechin; 4, chlorogenic acid; 5, methyl gallate; 6, mangiferin; 7, 4-hydroxybenzoic acid; 8, vanillic acid; 9, ethyl gallate; 10, *p*-coumaric acid; 11, ferulic acid; 12, rutin; 13, ellagic acid; 14, morin; 15, daidzein; 16, kaempferol.

Table 1. Sixteen Phenolic Compounds Identified by HPLC−ESI-MS and Their Intensity in Peel of *Mangifera pajang* Kort.[a]

peak no.	t_R, min	compound	UV λ_{max}, nm	MW	$[M + H]^+$ (m/z)	$[M - H]^-$ (m/z)	relative abundance
1	12.4	gallic acid	218	170.12	nd	169.07	3.3×10^6
2	15.4	protocatechuic acid	220, 280	154.12	nd	153.00	9.4×10^6
3	15.9	catechin	231, 280	290.2	nd.	289.15	7.2×10^6
4	16.4	chlorogenic acid	225, 244, 335, 396	354.31	355.07	353.02[b]	1.1×10^7
5	16.7	methyl gallate	220, 322	184.04	nd	183.06	1.4×10^6
6	17.5	mangiferin	241, 317, 365	422.33	423.06[b]	421.00	1.0×10^7
7	17.8	4-hydroxybenzoic acid	216, 253	138.12	139.11[b]	137.05	1.7×10^6
8	18.2	vanillic acid	220, 260, 280, 322	168.04	168.15	167.06[b]	8.5×10^5
9	18.7	ethyl gallate	220, 322	198.17	nd	197.00	5.3×10^6
10	19.8	*p*-coumaric acid	228, 275, 324	164.16	nd	163.08	6.2×10^6
11	20.4	ferulic acid	220, 322	194.18	nd	193.03	1.3×10^7
12	20.6	rutin	220, 256, 355	610.16	611.13[b]	609.07	6.6×10^6
13	21.7	ellagic acid	219, 373	302.01	nd	301.10	2.9×10^6
14	22.6	morin	220, 252, 353	302.04	303.02[b]	301.00	4.1×10^6
15	22.9	daidzein	248, 301	254.04	255.05[b]	253.06	1.2×10^8
16	24.8	kaempferol	221, 265, 365	286.05	287.02[b]	285.07	4.5×10^7

[a] nd: not detected in signed mode. [b] Highest compound intensity in signed mode.

280 nm and the bandwidth was 9 nm. A reversed-phase Lichrospher C18 column (250 mm × 4 mm, i.d., 5 μm, Merck, Darmstadt, Germany), at temperature of 30 ± 1 °C, and a gradient elution program were employed to confirm the identification of the selected analytes in real samples by HPLC−ESI-MS. Samples were run using a gradient elution system with 0.2% (v/v) acetic acid in deionized water as solvent A and with methanol (100%) as solvent B. The flow rate was 0.1 mL/min, and the gradient program was as follows: for the first 20 min, the linear gradient of solvent A dropped from 95% to 10% while solvent B increased from 5% to 90%, followed by 5 min with no shifts in buffer concentrations. The final step was a linear increased from 10% to 95% for solvent A and a decrease from 90% to 5% for solvent B. A reversed-phase Lichrospher C18 column (250 mm × 4 mm, i.d., 5 μm, Merck, Darmstadt, Germany), at temperature of 30 ± 1 °C, and a gradient elution program described above were employed to confirm the identification of the selected analytes in real samples by HPLC−ESI-MS. In the MS analysis (negative and positive full scan modes), data were collected over a mass of 100−800 m/z. The phenolic compounds in BPP were identified by comparison of their retention times, UV−vis absorption spectra, and MS with authentic standards.

Statistical Analysis. Unless otherwise stated, all of the experimental results were expressed as the mean (standard deviation of three determinations). SPSS (version 19.0 software, Chicago, IL, USA) was used for the statistical analysis. A one-way ANOVA was performed on the mean values to determine whether they differed significantly. Variables with r close to 0 indicate no linear relationship, whereas r values that are close to 1 suggest a strong linear relationship. Significant difference ($p < 0.05$) between total polyphenol content determined by

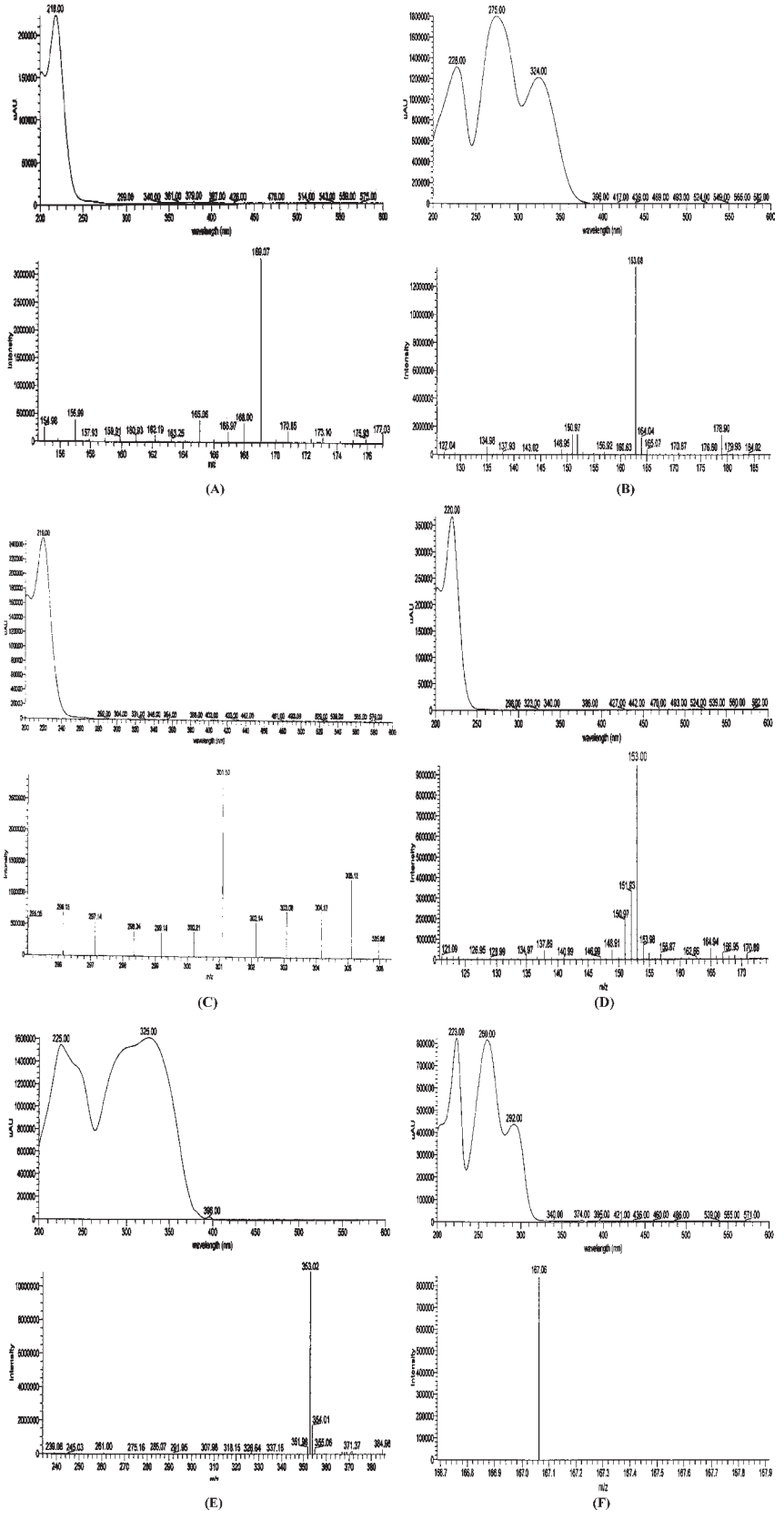

Figure 3. Continued

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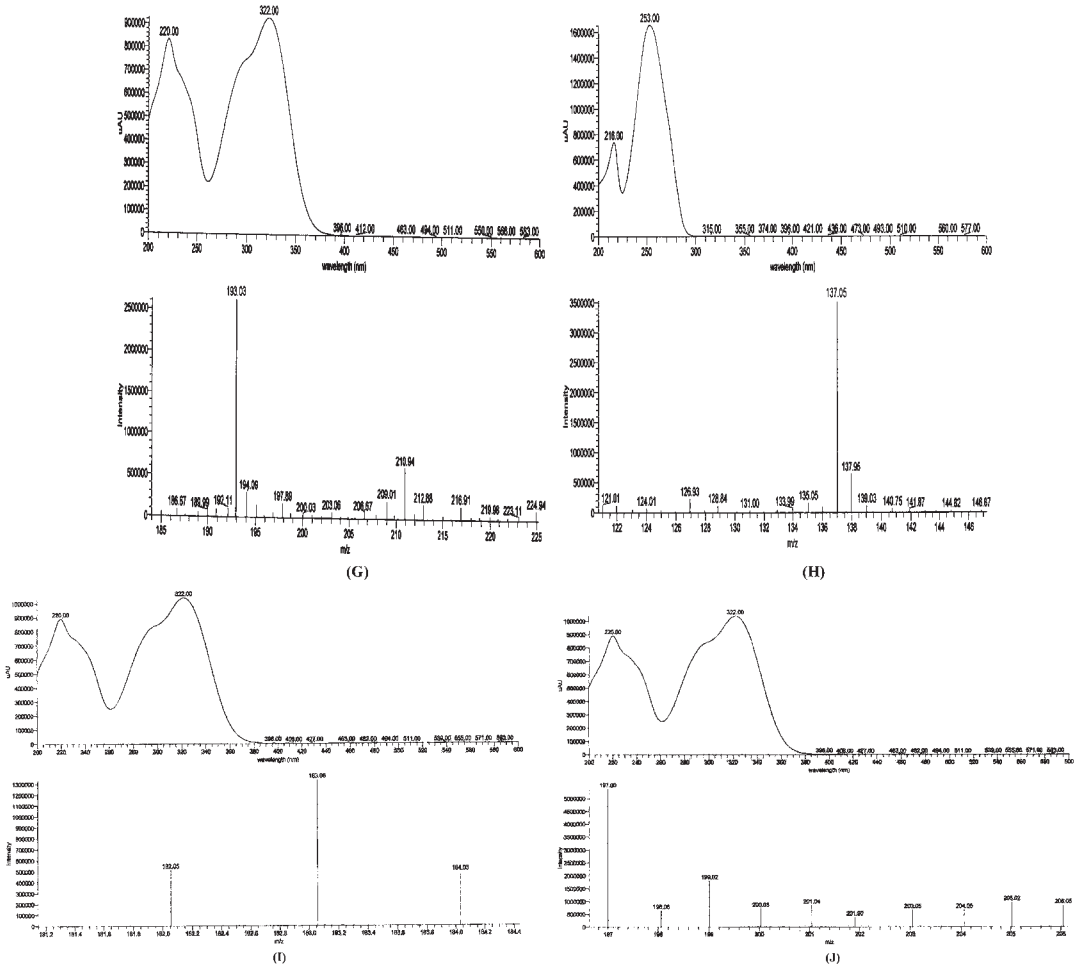

Figure 3. LC−ESI-MS spectra with UV−vis spectra LC-DAD−MS acquired in negative and positive full-scan mode corresponding to the $[M - H]^-$ and $[M + H]^+$ ions of each identified phenolic acid extracted from the peel of *Mangifera pajang* fruit. ESI-MS conditions are as in Materials and Methods. Identification of peaks is as follows: (A) gallic acid; (B) *p*-coumaric acid; (C) ellagic acid; (D) protocatechuic acid; (E) chlorogenic acid; (F) vanillic acid; (G) ferulic acid; (H) 4-hydroxybenzoic acid; (I) methyl gallate; (J) ethyl gallate. For assignment of structures, see Figure 1.

TPA and that quantified by HPLC was evaluated by one-way ANOVA using Tukey's multiple-comparisons test.

RESULTS AND DISCUSSION

Identification of Phenolic Compounds in Peel Extract. The prepared *M. pajang* peel extracts were analyzed for phenolic acids, flavonoids, and xanthone. The extract was analyzed by reverse-phase HPLC, and 16 major phenolics were identified: gallic acid (**1**), protocatechuic acid (**2**), (+)-catechin (**3**), chlorogenic acid (**4**), methyl gallate (**5**), mangiferin (**6**), 4-hydroxybenzoic acid (**7**), vanillic acid (**8**), ethyl gallate (**9**), *p*-coumaric acid (**10**), ferulic acid (**11**), rutin (**12**), ellagic acid (**13**), morin (**14**), daidzein (**15**), and kaempferol (**16**) (Figure 2), with retention times of 12.4, 15.4, 15.9, 16.4, 16.7, 17.5, 17.8, 18.2, 18.7, 19.8, 20.4, 20.6, 21.7, 22.6, 22.9, and 24.8 min, respectively (Table1) .

Efforts to identify these peaks by comparing the retention times of the authentic standards or spiking with known phenolics did not help in identifying the peaks, as there were little variations with retention times of many of the compounds due to the complex nature of polyphenols. However, complete identifications could not be made in all cases. Accordingly, more structural information obtained using LC−MS is critical for authoritative identification. To confirm the results of HPLC identification, the structures and molecular formulas of the 16 phenolic compounds (Figure 1) were determined from their LC−ESI-MS spectra.

Phenolic acids are classified as free, soluble, or insoluble bound phenolic acids.[21] Most of the phenolic acids occur as esters, acetals, or ethers; some are connected to the structural components of plants such as proteins, fibers, glucose, or other natural products.[22] In this study, the extract was hydrolyzed to disconnect the conjugated phenolics. The free phenolics were separated by HPLC on a reverse phase C18 column. The eluted compounds were read at 280 nm (Figure 2). The retention times of these peaks were compared with the authentic standards of phenolic acids. When there were variations in retention times, peak identifications were supported by spiking with authentic standards and confirmed by LC−ESI-MS analysis.

Phenolic compounds were further identified by LC−MS. The identification of the peaks detected under full-scan conditions was obtained by analyzing the extracted-ion chromatograms of the ion current at m/z values corresponding to the $[M - H]^-$ and the $[M + H]^+$ ions of the studied compounds. Typical UV spectra and MS data for the phenolic compounds are shown (Table 1). The identities of all compounds were confirmed by

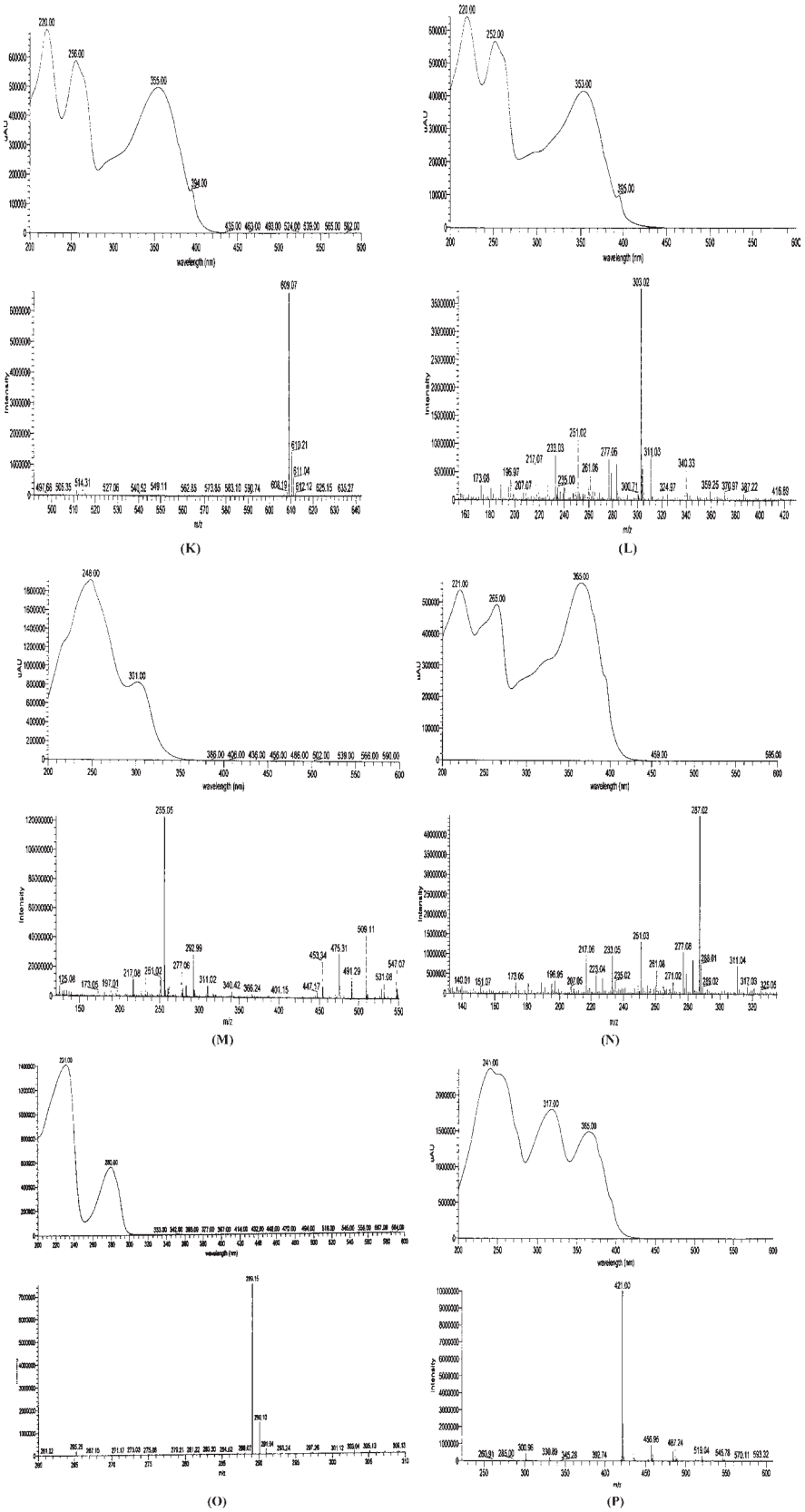

Figure 4. LC−ESI-MS spectra with UV−vis spectra and LC-DAD−MS results acquired in negative and positive full-scan modes corresponding to the $[M - H]^-$ and $[M + H]^+$ ions of each identified flavonoid and xanthon extracted from the peel of *Mangifera pajang* fruit. ESI-MS conditions are as in Materials and Methods. Identification of peaks is as follows: (K) rutin; (L) morin; (M) daidzein; (N) kaempferol; (O) catechin; (P) mangiferin. For assignment of structures, see Figure 1.

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Table 2. Quantities of Phenolic Compounds in *Mangifera pajang* Peel Using HPLC-DAD

compound	regression equation[a]	correlation coefficient	amount BPP,[b] mg/g
gallic acid	$y = 69.833x + 197.52$	0.9993	20.93 ± 0.3
p-coumaric acid	$y = 87.467x + 299.64$	0.9959	12.65 ± 0.14
ellagic acid	$y = 3.735x + 433.3$	0.9912	7.32 ± 0.05
protocatechuic acid	$y = 52.203x + 116.64$	0.9979	5.4 ± 0.08
mangiferin	$y = 83.324x - 36.381$	0.9982	4.75 ± 0.04
rutin	$y = 23.167x + 52.143$	0.9947	3.78 ± 0.08
catechin	$y = 35.451x - 60.09$	0.9979	3.4 ± 0.06
kaempferol	$y = 12.185x + 10.4$	0.9991	1.75 ± 0.04
ethyl gallate	$y = 85.334x + 438.9$	0.9953	0.9 ± 0.03
methyl gallate	$y = 86.64x + 311.3$	0.9936	0.65 ± 0.02
ferulic acid	$y = 21.769x + 46.571$	0.9969	0.6 ± 0.01
vanilic acid	$y = 92.421x + 123.76$	0.9981	0.43 ± 0.01
morin	$y = 25.636x + 41.048$	0.9979	0.4 ± 0.01
daidzein	$y = 74.03x + 124.6$	0.9967	0.38 ± 0.01
chlorogenic acid	$y = 55.228x + 197.36$	0.9959	0.3 ± 0.01
4-hydroxybenzoic	$y = 222.13x + 775.25$	0.9985	0.18 ± 0.01
Total			63.82 ± 0.9

[a] y expresses the detection response (peak area mAU) and x the concentrationof phenolic compound (in μg/mL). [b] Amount is the mean value ± standard deviation; $n = 3$.

comparison of the data with those obtained for standard compounds. The corresponding UV−vis and MS data for the 16 compounds identified were used for structural characterization.

The identification of the phenolic compounds in the mixture of bambangan peel extracts was first performed based on their retention times and the UV spectra acquired in the wavelengths between 100 and 800 nm with a photodiode array (PDA) detector (Figure 3 and Figure 4). Peak identifications were confirmed by ESI-MS detection in a single ion monitoring (SIM) mode in connection with PDA detection, resulting in the appearance of signals at m/z values corresponding to the main ion of the selected analytes in correspondence with the peaks seen on the PDA chromatogram.

Table 1 tabulates the m/z values of the major ions observed for each analyte at optimized values of applied voltages at the three-stage high-frequency lens system (Q-array). The utilization of a narrow-bore column allows one to work with a small sample volume and a low flow rate applied to the ESI interface. ESI has recently been demonstrated to be a powerful tool for the identification and characterization of phenolic compounds by online MS detection.[23] Optimum ESI-MS analysis conditions were obtained by the flow injection analysis of standard solutions of the analytes at concentrations ranging between 100 and 1000 ppb. In the present study, the phenolic acids (except for 4-hydroxybenzoic acid) were detected in ESI (−) mode at better sensitivity and lower background noise compared to the ESI (+) mode (Table 1; Figure 3). In contrast, the flavonoids (except for catechin) were more effectively detected in the ESI (+) mode than the ESI (-) mode (Table 1 and Figure 4).

Quantitative Analysis of Phenolic Compounds. The quantitative analysis of the phenolic compounds in bambangan peels was performed by HPLC. The concentrations of each compound in the studied extract were calculated from the corresponding standard curve and were arranged in descending order as follows: gallic acid > *p*-coumaric acid > ellagic acid > protocatechuic acid > mangiferin > rutin > catechin > kaempferol > ethyl gallate > methyl gallate > ferulic acid > vanillic acid > morin > daidzein > chlorogenic acid > 4-hydroxybenzoic acid (Table 2).

Phenolic Acids. In addition to their antioxidant and anticancer activities, a study reported that phenolic acids such as gallic acid and methyl gallate have antimicrobial activity, controlling dental caries and periodontal disease.[24] Other studies have reported that ellagic acid exhibits antimutagenic, antiviral, antitumor, and antioxidant properties, along with the ability to whiten the skin.[25]

In the current study, gallic acid was the major component, at 20.93 mg/g dry weight, followed by *p*-coumaric acid at 12.7 mg. Ellagic acid was third (7.32 mg). The fourth major compound was protocatechuic acid (5.4 mg). The other six phenolic acids (ethyl gallate, methyl gallate, ferulic acid, vanillic acid, chlorogenic acid, and 4-hydroxybenzoic acid) were present at 0.9, 0.65, 0.6, 0.43, 0.30, and 0.18 mg/g dry weight, respectively (Table 2).

Flavonoids and Xanthone Aglycone. The quantitative analysis of individual flavonoid glycosides in plant materials is complicated because of their large number. Consequently, the glycosides are usually hydrolyzed and the resulting aglycones are identified and quantified.[19] Flavonoid compounds in *M. pajang* extract amounted to 9.7 mg/g dry weight as follows: 3.78 mg/g dry weight for rutin, 1.7 mg for kaempferol, 0.4 mg for morin, and 0.38 mg for daidzein. Catechin, a flavanal, was present at 3.4 mg/g dry weight. Among these compounds. xanthon (mangiferin) was the fifth major compound that has been found in *M. pajang* peels at an amount of 4.8 mg/g dry weight (Table 2). This compound has a wide range of pharmacological effects including hypolipidemic, antidiabetic, anti-HIV, antitumor, immunomodulatory, and antioxidant activities.[26−28] Naczk and Shahidi [29] mentioned in their review that mango peels are rich with xanthones and flavonols.

The sum of all phenolic compounds concentrations was 64 mg/g dry weight (Table 2).

Total Phenolic Content (TPC). The average BPP content of TPC expressed as gallic acid equivalents was 98 mg/g dry sample (Table 3). This value was in agreement with values for *Mangifera indica* ripe peel reported previously[30] but was greater than that of Hayden mango peel fiber (70 mg/g).[31] Thus, BPP is rich in

Table 3. Radical Scavenging Activity (RSA) and IC_{50} of Bambangan Peel Powder (BPP) Using BHT as Reference

sample	TPC[a,c]	IC_{50},[b,c] μg/mL	concn, μg/mL	RSA, %
BPP	9.8 ± 0.12	44.5 ± 0.24	250	97
			200	90
			150	86
			100	82
			50	57
BHT		17 ± 0.11	100	89

[a] Total phenolic content (TPC) is expressed as (g of GAE)/(100 g of BPP). [b] Inhibition concentration that is from the curve of RSA and concentration of BPP. [c] Values are the mean of triplicates ± standard deviation.

polyphenolic compounds. A significant difference ($P < 0.05$) was observed between the total polyphenol content of bambangan peel extract, as shown in Table 3, and that calculated by the sum of the individual phenolic compounds obtained using the HPLC (64 mg/g dw). The overestimation of phenolic content by the Folin−Ciocalteu method could be due to interference from sugars and protein,[32] overlapping of spectral responses,[29] or the presence of additional phenolic compounds that were undetectable by our HPLC method.

DPPH Radical-Scavenging Activity (RSA). Several methods to determine free radical scavenging have been reported, with assay times varying from 10 to 20 min up to approximately 6 h.[33] In the current study, samples of BPP had high DPPH radical-scavenging activity, ranging from 57% to 97% (Table 3). At 250 μg/mL, the extract scavenged more than 97% of the DPPH radical, compared to the BHT control. The BHT control was assayed at 100 μg/mL, corresponding to the maximum allowable concentration for BHT addition to foodstuffs.[34] The BPP extract showed higher RSA values at 200 and 250 μg/mL than BHT (Table 3). Values between 50 and 250 μg/mL were used to determine the inhibition concentration (IC_{50}), which is the amount of antioxidant required to deplete the initial [$DPPH^{\bullet}$] by 50%. The IC_{50} of the extract was 44 μg/mL compared to 17 μg/mL for BHT (Table 3). The greater ability of the extract to donate electrons may be attributed to a more elevated level of phenolics reflected in the increase in total phenolic content (TPC). It was observed that the phenolic content of *M. pajang* extract is associated with an increased radical-scavenging capacity. This effect may be due to the electron-donating ability of the phenolic compounds.

The current study used a hydrolysis method to release the phenolic compounds, thus improving the antioxidant capacity of the extract. Soong and Barlow[25] demonstrated that the shift from the conjugated form to the free form during hydrolysis of gallic acid and ellagic acid increased antioxidant capacity and improved the correlation between concentration and antioxidant capacity.

FRAP Assay. The principle of FRAP assay is based on the capacity of reducing the TPTZ−Fe(III) complex to the TPTZ−Fe(II) complex, forming an powerful blue Fe^{2+}−TPTZ complex at maximum absorption of 593 nm.

The antioxidant capacities of the extract and vitamin C as control are shown in Figure 5. The BPP exhibited a high antioxidant power corresponding to that of vitamin C. At the highest concentration the antioxidant power was 1248 μg/mL compared to that for vitamin C (1318 μg/mL). The result was higher than that reported for unripe bambangan peel.[1] This could be a result of the full ripening stage in the current study.

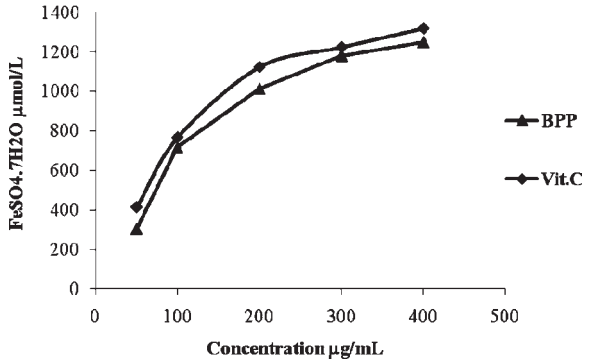

Figure 5. Ferric-reducing antioxidant power (FRAP) of bambangan peel powder (BPP) vs vitamin C.

In conclusion, the present study indicates that an extract of peels from *M. pajang* fruits is a rich source of polyphenols. Gallic acid, *p*-coumaric acid, ellagic acid, mangiferin, and protocatechuic acid were the major phenolic compounds identified. All these phenolic compounds were shown to exhibit antioxidant properties. In addition to its phytochemical content, we have recently reported that *Mangifera pajang* peel is rich in dietary fibers (DF), with a balanced ratio of soluble and insoluble DF.[17] This justifies the potential use of the peel as a nutraceutical.

As bambangan is a seasonal fruit, future work on its peel extract can be applied in pharmaceutical applications and the huge residue of peel that remains after the removal of bioactive compounds can be used as a functional food. Because this study obtained only small amounts of flavonoids, optimization of the extraction method needs to be carried out.

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